In vitro metabolism of neostigmine and pyridostigmine

Differences between the elimination of unchanged neostigmine and pyridostigmine in man (Nowell, Scott & Wilson, 1962) suggest that there may be quantitative or qualitative differences in the hepatic metabolism of these carbamates. We have examined this possibility by studying the metabolism of the two quaternary amines in *in vitro* conditions.

Male Wistar rats were killed by decapitation and the liver was removed and weighed. Liver homogenates were prepared in ice-cold sucrose (2 vol; 0·25M) and non-microsomal particulate matter was removed by ultracentrifugation (15 000 g; 15 min) and discarded. The remaining supernatant was separated into microsomal and soluble fractions (105 000 *g*; 60 min). Both fractions were incubated at 38° for 60 min with [^{14}C]neostigmine iodide (71·4 nmol in 0·05 ml) or [^{14}C]pyridostigmine iodide (81·2 nmol in 0·05 ml) and $NADPH_2$ (1 μ mol) in phosphate buffer (0·15 M, pH 7·4; 0·25 ml). In some experiments, fractions were heated (60° for 15 min) or dialysed (100 vol isotonic sucrose at 5°). After incubation, [^{14}C]neostigmine and [^{14}C]pyridostigmine were separated from their phenolic metabolites by paper electrophoresis (Somani, Roberts & Wilson, 1972) and the protein content of liver fractions was determined by standard methods (Lowry, Rosebrough & others, 1951).

As found by Roberts, Thomas & Wilson (1968), neostigmine was hydrolysed by liver microsomes, and its microsomal metabolism was greatly enhanced by the presence of the co-factor $NADPH_2$ (Table 1). In contrast, the hydrolysis of pyridostigmine was mainly dependent on the soluble fraction of rat liver. Metabolism by microsomal preparations was insignificant, and could well be due to contamination of the unwashed microsomes by the soluble faction. $NADPH_2$ had little or no effect on hydrolysis (Table 1). Pyridostigmine metabolism appears to be dependent on an enzyme-catalysed reaction, since hydrolysis was negligible when the soluble fraction was heated and was unaffected by dialysis before incubation.

Table 1. *Metabolism of neostigmine and pyridostigmine by the soluble fraction and the microsomes of rat liver.* Values represent the mean ± standard error of at least ten experiments.

	Mean activity (nmol quaternary amine hydrolysed $g^{-1}h^{-1}$)	
	Neostigmine	Pyridostigmine
Soluble fraction	1·3±0·5	15·2±1·4
Soluble fraction + $NADPH_2$	—	18·2±1·8
Microsomal fraction	0·7±0·3	0·3±0·2
Microsomal fraction + $NADPH_2$	125·9±26·8	2·1±0·7

These experiments suggest that there are qualitative differences in the metabolism of neostigmine and pyridostigmine by liver cells. Thus, microsomal enzymes are responsible for the metabolism of neostigmine; in contrast, pyridostigmine hydrolysis predominantly occurs in the soluble fraction of the liver cell, and is not dependent on the co-factor $NADPH_2$. Since hydrolytic activity was heat labile and non-dialysable, an enzymic process appeared to be involved. Differences between the duration of action of the two quaternary amines in man may well be related to these qualitative differences in drug metabolism.

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Resistance of rats to the potentiating action of phosphatidyl serine on dextran responses

One of the amines released from mast cells during the dextran anaphylactoid reaction in rats, is histamine and yet only trace amounts of this amine are released when isolated peritoneal mast cells are incubated with dextran (Selye, 1968). Recently, Goth, Adams & Knoohuizen (1971) reported that phosphatidyl serine (PS) selectively and markedly potentiates this histamine release from isolated mast cells and the potentiation has since been shown to be calcium-dependent, maximal enhancement occurring at physiological levels of 1·0 mM calcium when the concentration of PS is 10 μg ml^{-1} (Foreman & Mongar, 1972).

As a colony of Wistar rats genetically resistant to the dextran anaphylactoid reaction was available (Harris, Kalmus & West, 1963), it was of interest, therefore, to study the effect of PS on their isolated peritoneal mast cells as a deficiency in PS may be the cause of resistance to dextran. Furthermore, a study of the effects *in vivo* of PS on dextran in Wistar rats sensitive to dextran (ASH) as well as in Wistar rats resistant to dextran (NR) was considered important as the histamine release *in vitro* is so markedly potentiated.

Rats were anaesthetized with ether and decapitated. Four ml of Tyrode solution containing heparin (10 μg ml^{-1}) was injected intraperitoneally and after 1 min of abdominal massage the fluid containing the peritoneal mast cells was removed. Aliquots were diluted with Tyrode solution containing heparin (10 μg ml^{-1}) and different concentrations of dextran (average molecular weight 67 000), with or without PS (10 μg ml^{-1}).* After incubation at 37° for 15 min, the cells were separated from the fluid using Millipore filters, the protein in the fluid was precipitated using trichloroacetic acid and heat (100°, 10 min), and the precipitate was removed by centrifugation (1000 *g*, 5 min). Samples were then neutralized with NaOH and assayed using the fluorimetric method of Anton & Sayre (1969). To obtain the total histamine content of control samples, the above procedure was followed, except that the passage through Millipore filters was omitted. Values shown for histamine release have been corrected by subtraction of the spontaneous release.

Local anaphylactoid reactions were produced in rat paws by injecting 0·1 ml (s.c.) Tyrode solution containing either dextran or dextran with PS, and assessed by measuring the increase in volume 1 h later using a volume differential meter. Other animals were injected intradermally with 0·1 ml Tyrode solution containing either dextran or dextran with PS into the shaved skin of the back after pre-treatment with intravenous azovan blue dye (30 mg kg^{-1}). 30 min later, they were killed and the reaction assessed by measuring the amount of dye in each weal using the method of Harada, Takeuchi & others (1971).

* (Koch-Light, Folch fraction 3.)